Chromosome Aberrations and Their Relevance to Metal Carcinogenesis

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Scoring for structural chromosome abnormalities is one of the only practical methods available for detecting visual damage in human genetic material. Cytogenetic tests *in vivo* and *in vitro* have shown the clastogenic potential of a number of metals and metal compounds. The difficulties in *in vivo* studies lie in identifying a specific clastogen in an occupational setting, where simultaneous exposure to a number of organic and inorganic chemicals is a common phenomenon. Metals known to be carcinogens in animals also tend to possess chromosome-damaging properties, even though more extensive studies are needed before any conclusive evidence can be reached. The visible chromosomal damage produced by exposure to metal compounds should be considered as a warning indication of potentially adverse genetic and somatic effects in humans.

Today genotoxic effects of environmental agents have high priority in research related to public health. Alterations in genetic material are undoubtedly significant in the etiology of cancer and congenital malformations. Since experimental assay systems capable of revealing changes in genetic material are more sensitive, quicker, and easier to run than tests depending upon tumor formation or teratological findings in laboratory animals, increasing emphasis has recently been placed on the assessment of mutagenicity.

Interaction between chemicals and genetic material results principally in two types of deoxyribonucleic acid (DNA) alterations: first, changes in single genes at the molecular level and, second, chromosomal aberrations derived from breakage in the linear coherence of chromosomes. Another type of genetic alteration may be induced by chemicals affecting the segregation of chromosomes through interaction with the spindle fiber mechanism, the result being nondisjunction and a deviant number of chromosomes.

In addition to short-term test systems, cytogenetic methods applied directly to human cells can be used to evaluate genotoxic hazards. Cytogenetic analysis for chromosome aberrations in risk groups exposed to radiation or chemical hazards provides an important tool for detecting possible, environmentally caused genetic damage in man. Cytogenetic monitoring is becoming an integral part of preventive medical surveillance in occupations with actual or potential genetic hazard (1).

Although a large number of metals have been reported to be carcinogenic in experimental animals (2), the molecular mechanism of metal carcinogenesis has been, and still is, under debate. The present article attempts to synopsize current knowledge of the chromosomal effects caused by metal exposure, point out the existing gaps in this knowledge and discuss future research needs.

Methods for Detecting Somatic Chromosome Damage

Cytogenetic techniques as visual means of detecting genetic damage are very important, partly because of the formidable technical, financial, and political difficulties for programs on induced human germinal mutation frequency. The following methods are currently available for observing somatic chromosome damage in exposed subjects: analysis of metaphase chromosome aberrations, scoring for sister chromatid exchanges (SCEs), and detection of micronuclei in maturing erythrocytes.

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Metaphase Chromosome Aberrations

The cell system most frequently used is mitogen-stimulated lymphocytes in short-term cultures of a blood sample taken from subjects by venipuncture (3). Because of their long lifespan, the use of phytochemagglutinin (PHA) stimulated T-lymphocytes offers the possibility of detecting accumulated damage. Especially with chemical mutagens (which represent a wide variation in the mode and specificity of action) the persistency of damage, the specificity of the cellular stage responsive to the mutagenic action, and the possible selectivity of cells responding to mitogen stimulation are bound to cause uncontrolled variability in the results.

Consequently, real in vivo detection of damage can only be obtained from direct samples of dividing human cells, e.g., from bone marrow or testicular tissue. The use of such tissues has obvious disadvantages, because of difficulties in obtaining the sample, and such techniques can only be applied in special cases with strong indications of a genetic health hazard.

Scoring for Sister Chromatid Exchanges

In addition to the conventional cytogenetic parameters observable in metaphase chromosomes (mitotic disturbances, incompleteness of chromosomal coiling and chromosome breakage and rearrangements), recent developments in chromosome staining methods offer the possibility of observing exchanges between sister chromatids (4). Even though the exact molecular mechanism of exchange is not yet certain, it is evident that SCE is a sensitive reflection of damage to DNA (5).

The harlequin staining method, based on the evaluation of the frequency of SCEs, can be applied in vitro to both human lymphocytes exposed in vivo (6) and bone marrow cells (7) with the aforementioned restrictions. The efficiency of SCE testing in chronically exposed populations still needs to be established even though available evidence suggests that, e.g., solvent exposure in laboratory work (6) and smoking (8,9) increase the SCE frequency in lymphocytes.

The correlation between an increased frequency of SCEs and carcinogenesis may be derived from the fact that SCEs represent a form of somatic recombination, a mechanism which may convert mutations induced by initiator carcinogens into expression (e.g., recessives into the homozygous condition). Therefore, much interest has recently been focused on whether typical cancer-promoting agents, e.g., phorbol esters, would induce SCEs. This phenomenon has, in fact, been observed (10),

even though contradictory evidence is also available (11). However, in members of families with high cancer incidence, SCE frequency has not been found to be higher than normal (12).

Scoring for Micronuclei

The microscopically observable micronuclei in maturing erythrocytes are nuclear fragments resulting from chromosome breakage or chromosome losses (13). The inducing agents can be either clastogens or spindle-inhibiting agents affecting the normal segregation mechanism of chromosomes during karyokinesis.

In human material, genetic damage observable as micronuclei in polychromatic erythrocytes can only be observed in bone marrow. The observations are therefore few. Recent findings suggest that in bone marrow samples from patients under cytostatic therapy, the formation of micronuclei may be a more sensitive indicator of genetic damage than clastogenicity in metaphase chromosomes (14,15).

Biological Significance of Somatic Chromosome Aberrations

Genetic Effects

Visible chromosome damage in a sample of somatic cells taken from an exposed individual provides a direct indication of exposure to clastogenic agents. Even though the unrepaired damage in the indicator cells may be lethal to the cell, and thus of no significance as such, especially in the case of normally nondividing T-lymphocytes, the observation suggests that alterations of genetic material may also have occurred in cells of other tissues.

Experimental evidence also closely relates chromosome breakage and clastogenic agents to molecular point mutations and agents causing DNA lesions (16,17). Thus visible damage also indicates that invisible lesions may have occurred. Such microlesions may have an even higher probability for being transmitted in cell proliferation and thus for being manifested.

The significance of chromosome mutations inherited in the germinal cell line has clearly been documented in relation to spontaneous abortions (30-50%) and live births with chromosome anomalies (0.5-0.7%) (18). However, as yet, there is no clear evidence that a population exposed to chromosome breaking agents would show an increased incidence of constitutional chromosomal or genic abnormalities among their offspring. The difficulties involved in carrying

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out such studies should, however, not diminish the concern about the health of future generations.

Neoplasia

It was as early as 1914 when Boveri published a theory in which he argued that the fundamental cause of tumors is an imbalance in the chromatin content of the cells from which they arise. Through the development of chromosome banding techniques, data on chromosomal changes in malignancies has increased enormously since then. It has become evident that neoplasms are often of monoclonal origin and that the chromosomal changes frequently found in tumor tissue tend to have a nonrandom character (19,20). The best-documented correlation of a specific chromosomal change with human malignancy is the characteristic Ph^1-chromosome (material deleted from chromosome 22 is usually translocated to chromosome 9) in about 90% of the patients with chronic myeloid leukemia. The clustering of karyotypic alterations in specific human chromosomes in neoplasms suggests that such changes provide the mutant cell with a proliferative advantage and thus give rise to a malignant clone.

The role of chromosomal etiology in human cancer proneness is also supported by correlations of high chromosomal instability—both "spontaneous" and induced—and an increased risk of malignancies in such human hereditary disorders as xeroderma pigmentosum, ataxia telangiectasia, Bloom's syndrome, and Fanconi's anemia (21).

Immunologic Impairment and Aging

Impaired immunocompetence is a well known side effect in patients under cytostatic or irradiation therapy. Depressed humoral and/or cell-mediated immune responses have been experimentally observed in rodents exposed to different toxic agents, also to lead, cadmium, mercury, and organic tin (21-24). Even though much research is presently oriented towards the characterization and definition of the mechanisms responsible for immunosuppression, only vague guesses can be made about the role of genetic damage in the lymphocytes responsible for immunocompetence. The possible association of immunologic defects with some mutagen sensitive human genetic diseases showing increased rates of chromosome aberrations in peripheral blood lymphocytes (25) point to a close correlation. To our knowledge no attempts have been made to relate possible changes in immune response reactions in populations with high chromosome aberration rates due to exposure to clastogenic and possible lymphocytotoxic agents.

Similarly, it can only be speculated as to whether or not the significance of genetic damage (due to failure of repair functions) in somatic cells incites the process of aging. It would be logical to suppose that accumulated somatic mutations underly normal aging development also at the level of the whole individual, as has been exhibited to occur in human cell cultures (26). Supporting evidence is provided by some hereditary, premature aging syndromes characterized by cellular sensitivity to agents that damage DNA (25).

Chromosome Abnormalities in Human Populations Exposed to Metal Compounds

Chromosome aberration tests *in vivo* and *in vitro* in mammalian cells have demonstrated the chromosome breaking capacity of numerous inorganic chemicals. Very few studies exist, however, on the effects of metal compounds on the frequency of SCEs or micronuclei. Therefore, we have concentrated on published data concerning increased chromosome aberrations in exposure to metals, and even then the number of studies is few. The results of *in vivo* and *in vitro* studies with the same test system, human lymphocytes, sometimes disagree (Table 1). This inconsistency may be due to differences in concentrations at target sites on one hand, or to the capacity of the body to eliminate injured cells *in vivo* on the other.

Arsenic

Arsenic is a well known human carcinogen (2). Also most of the studies performed on the mutagenic activity of arsenic have provided positive results (27). An increased frequency of chromosome aberrations has been found in the peripheral lymphocytes of wine growers, in psoriatic patients treated with arsenic (28), and in arsenic-exposed workers from a copper smelter (29). In the last-mentioned study an enhancement of chromosome aberrations was observed due to the interaction of smoking and arsenic exposure.

Cadmium

Cadmium compounds have been shown to induce local sarcomas and testicular tumors in rodents (2). There are also some indications that occupational exposure to cadmium may be connected with increased cancer frequency of the prostate, lungs and other organs (30).

It is still an open question as to whether cadmium can cause chromosome aberrations in the lymphocytes of people occupationally exposed to cadmium.

Table 1. Chromosomal aberrations in human lymphocytes in *in vivo* and *in vitro* exposure to metal compounds and their carcinogenicity in rodents.[a]

Test system	Metals											
	As	Be	Cd	Co	Cr	Cu	Hg	Mn	Ni	Pb	Pt	Zn
Chromosomal aberrations in human lymphocytes in *in vivo* exposure	+ (28) (29) (62)	..	− (33) (34)	..	+ (38)	..	+ (47)	..	..	+ (49, 51 53-55, 66) − (59, 60)	..	+ (61)
Chromosomal aberrations in human lymphocytes cultured *in vitro*	+ (63) (64)	− (64)	− (44) (64)	− (64)	+ (43) (44) (65)	..	− (44)	+ (44)	+ (44)	+ (48)	..	+ (60)
Carcinogenicity in animal bioassays (2)	−	+	+	+	+	+	..	+	+	+	+	+

[a]Legend:
+ positive data, − negative data, .. no data available.

Markedly positive results have been obtained from Japanese Itai-Itai disease patients (*31,32*). Analysis of 72-hr lymphocyte cultures showed that the patients had a markedly increased frequency of aberrant cells (26.7% compared to 2.6% in controls). A question, however, has been raised as to whether the observed effects are due to cadmium or to diagnostic radiation (*33*). In addition Bui et al. (*34*) reported negative findings in lymphocytes of four persons with Itai-Itai disease and five male workers from an alkaline battery factory. Deknudt and Leonard (*35*) reported an increased incidence of "complex chromosomal aberrations" in a group of 23 men exposed to high levels of cadmium (B-Cd 3.2 μg/100 ml) and lead. Bauchinger et al. (*36*) also studied workers exposed to lead (mean B-Pb 19±7 μg/100 ml) and cadmium (B-Cd 0.40±0.27 μg/100 ml) and found an increase of both chromatid and chromosome type aberrations. Cadmium chloride has also been suggested to be a chromosomal mutagen in oocytes of the golden hamster *in vivo* (*37*).

O'Riordan et al. (*33*) studied a group of 40 men occupationally exposed to cadmium salts (mean blood cadmium level 1.95 μg/100 ml). A point of interest was the finding of four cells (out of 3740) containing chromatid interchanges in the exposed group but none in the control one. The authors' interpretation was that "the detection of four chromatid interchanges would, however, not be inconsistent with a small, but over-all imperceptible, aberration increase."

Chromium

Since the 1930's many surveys (*2*) have revealed that workers from various chromium industries have an increased risk of developing lung cancer. However, little is known about the possible clastogenic effects of chromium *in vivo*. Bigaliev and co-workers (*38*) reported an increase of chromosome aberrations in peripheral lymphocytes (from 5.98 to 9.97%, with 1.82 in control) in persons engaged in chrome production.

In cultured Chinese hamster cells chromate salts of both high and medium water solubility were active in producing both mutagenic and clastogenic changes, whereas an insoluble chromate salt (lead chromate) and a soluble chromic salt (chromic acetate) were inactive (*39*). Chromates are also known to be capable of inducing chromosome damage in cultured mammalian cells (*40,41*) and micronuclei in mouse bone marrow (*42*). Nakamuro et al. (*43*) reported chromosome breaking activity to be higher for compounds with hexavalent than trivalent chromium, the efficiency being, in decreasing order, $K_2Cr_2O_7 > K_2CrO_4 >> Cr(CH_3COO)_3 > Cr(NO_3)_3 > CrCl_3$. Umeda and Nishimura (*44*) also found hexavalent chromium compounds to be clastogenic in cultured mammalian cells.

Mercury

There is no evidence for the carcinogenicity of mercury or mercurials. In spite of the well-known harmful ecological consequences of mercury, very few studies are available on its chromosomal effects in exposed humans.

In persons exposed to methylmercury by eating contaminated fish, Skerfving et al. (*45,46*) observed a statistically significant increase of aneuploid cells. This phenomenon has been confirmed by Verschaeve et al. (*47*) who found that not only methylmercury, but also exposure to mercury vapor, caused an

increase in aneuploid cells. Supposedly the induction of aneuploidy results from the interaction of mercury compounds with the sulfhydryl groups of the spindle fiber.

Nickel

Epidemiologic studies have indicated that certain occupational exposures to nickel are associated with increased incidences of, mainly, respiratory and laryngeal cancers (2,30). However, no studies on the chromosome aberrations of nickel-exposed workers are known to us. In mammalian cell cultures, certain nickel compounds [$NiCl_2$ and $Ni(CH_3COO)_2$] have produced chromosome aberrations (44).

Lead

Numerous studies indicate that renal tumors may be produced by various inorganic lead compounds in rodents (30). The carcinogenicity of lead in humans is still an open question (48).

In the last few years a number of reports have been published on the chromosomal effects of lead in humans (49,50). Forni et al. (51), Deknudt et al. (52,53), Deknudt and Leonard (35), Bauchinger et al. (36), Sarto et al. (54), and Högstedt et al. (55) have all reported a positive correlation between blood lead levels and the percentage of chromosomal aberrations, whereas Bauchinger and Schmid (56), Bauchinger et al. (57), Schmid et al. (58), O'Riordan and Evans (59) and Bijlsma and DeFrance (60) did not find such a correlation.

Several explanations for the discrepancy have been suggested. The duration of cultures derived from lead-exposed people might be responsible for some of the variability between different reports. When lymphocytes are cultured for 72 hrs a large proportion of the cells are already in their second or third metaphase. Thus there is a possibility that some of the aberrations are not derived from *in vivo* exposure but develop during the culture period due to existing lead. However, Deknudt and Deminatti (61) reported that lead at a concentration of about 3300 times the concentration in the blood of occupationally lead-exposed workers did not produce chromosomal aberrations *in vitro* in human lymphocytes. There is, however, also a possibility that the lymphocytes with chromosomal damage tend to have a delay in their blast transformation, so that more first division metaphases are actually scored at 72 hr in lead-exposed subjects than normally.

Another factor which may explain some of the differences observed is the presence or absence of exposure to other factors either nutritional or environmental. It has been suggested that the lack of calcium is the principal reason for the chromosome aberrations found in lead intoxication (53). Occupational exposure to lead almost always also contains exposures to other metals such as arsenic, zinc, cadmium and copper, and thus the exact role of a single chemical is difficult to establish.

Some General and Methodological Problems

Intraspecies genetic variation in susceptibility to environmental agents is a known fact. Familial clustering of malignancies, inherited variation in the activities of various xenobiotic metabolizing enzymes, and polymorphisms of lipoproteins and other serum proteins is becoming increasingly evident as a background in the human health burden (67). Inherited susceptibility may also be responsible for variation in chromosomal responses to clastogenic agents. Variation in susceptibility may be partially overcome by cytogenetic monitoring at the individual level, i.e., prospective follow up of chromosomal changes in a risk occupation.

Methodological problems causing variability in results, e.g., gap/break scoring problems, time of lymphocyte culturing (48-72 hr), amount of metaphases analyzed and types of aberrations scored, can largely be overcome by interlaboratory cooperation and standardizations. This type of activity has already been begun and should be encouraged.

Assessment of the Health Implications of Chromosome Aberrations

When one considers the possible health effects of induced chromosome aberrations in man, perhaps the most important are the changes which functionally alter the cell but leave it capable of proliferation. Many chromosome aberrations will result in cell death, or at least lead to an inability to complete mitosis, i.e., reproductive killing. It is not only the number of aberrations produced, but also their specific location which is of concern. Irrespective of the development in techniques, we still know very little about the specificity of clastogens, or even whether it exists at all. Chromosomal banding techniques allow a better identification of minor chromosomal changes, especially translocations and inversions, but they are time-consuming and not applicable to routine tests. Structural aberrations are poorly resolved in molecular terms. With the

chromosome banding technique the detection of the deletion of a chromosome band is about the limit of aberration detection with a light microscope. Each band contains approximately 4.7×10^6 nucleotide pairs (68). Thus significant structural changes can occur within a band and remain wholly undetected by visual means. The idea of using visible changes as a marker actually points to the possibility that invisible changes may also have been produced.

Empirical data from Drosophila (69) suggest that chemical carcinogenicity may be better correlated with point mutations, i.e., with small deletions or lesions at the nucleotide level. The changes at the chromosomal level may nevertheless play an important role in carcinogenesis, for instance, in the promotion of the malignant potential of the transformed cell (10,19) through an expression of the initiated oncogenic factors of the genome.

At present we know that there is a rough dose-response relationship for radiation-induced chromosomal abnormalities and cancer incidence (70). Whether similar dose-response relationships also occur for chemicals is not yet certain. Benzene causes chromosome aberrations and is also a leukemogen (71,72). Vinyl chloride exposure is associated with an increased rate of chromosome aberrations and with an increased incidence of tumors in various organs (73).

Too little is known about the chromosomal effects of metal exposure. Even though the mechanism of metal clastogenesis may be different from covalently binding chemical mutagens, the biological significance of the induced damage should be considered to be the same. In health hazard evaluations chromosomal damage caused by metal exposure should act as a warning indicator for possible adverse somatic and genetic effects.

Although an individual prognosis cannot be derived on the basis of chromosome data, we may suspect that an individual with a prolonged increased level of somatic chromosome aberrations has been exposed to causative chemical concentrations that are too high. On an individual basis, increased somatic chromosome damage that is clearly above the level of the average of the group may also point to an inherited susceptibility of the individual. As yet, very little is known, e.g., of the role of repair efficiency in clastogenic responses.

We may conclude that a group of exposed persons showing increased levels of chromosome abnormalities has a higher risk of developing cancer than a group showing no increase in aberrations. The quantitative estimate of risk cannot, however, be deduced from the chromosomal data of a single individual.

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